ISSN : 0974 - 7435 Volume 5 Issue 4

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 5(4), 2011 [242-245]

Effect of some pesticides on the induction and growth of somatic embryos of *Solanum melongena* L.

D.Sammaiah[2]*, Ch.Chandra Shekar[2], M.Jaya Prakash Goud[1], K.Jaganmohan Reddy[2]

[1]Department of Botany, Kakatiya University, Warangal, (A.P.), (INDIA)

[2]Department of Microbiology, Kakatiya University, Warangal, (INDIA)

E-mail : ds.dasari@yahoo.com

Received: 25th April, 2011 ; Accepted: 25th May, 2011

ABSTRACT

In vitro somatic embryogenesis and regeneration was achieved from cotyledon explant of *Solanum melongena* L. Somatic embryogenesis by the addition of three different pesticides like Endosulfan and Rogor individually and in combination with Kitazin was studied at different concentrations of these chemicals in MS medium supplemented with 10 mg/l NAA. The investigation was carried out to study the induction, maturation, germination and regeneration of induced embryos in relation pesticides concentrations.

KEY-WORDS

Green roung brinjal;
Pesticides;
Somatic embryogenesis;
Regeneration.

INTRODUCTION

The recent developments in agro technology have accelerated the use of pesticides in an enormous amount. Pesticides are the modern tools to the farmers to control pests, diseases, weeds and to increase crop yields. A lot of work has been done on the role of pesticides in providing protection to plants against weeds in terms of crop yield[1-3]. Only a little work has been established on the role of pesticides in affecting biochemical characteristics of the plants[4].

Although tissue culture of *Solanum melongena* is being carried out for more than 25 years, reports regarding somatic embryogenesis in *Solanum melongena* has been reported earlier in hypocotyls and leaf segments[5,6]. The study of pesticides *in vitro* level provide a suitable source for morphogenetic developments and the analysis of molecular and biochemical events occurring during induction and maturation.

Hence, present study is an attempt to study the effect of 2 pesticides like Endosulfan, Rogor either alone and in combination with other fungicide Kitazin on the development of embryoids from the cotyledon derived embryogenic callus and their conversation into plantlets.

EXPERIMENTAL

Materials and Methods

Healthy and uniform seeds of *Solanum melongena* (L) was selected. Surface sterilization of the seeds was done with a commercial detergent teepol (Reckitt Colman, India) for 15 min, 0.1% Mercuric chloride for 10 min followed by washing four times with sterile distilled water to remove traces of $HgCl_2$. Seeds were

germinated in MS basal medium. Cotyledons from 8-10 days old was considered as the best explant for the studies on basis of the percentage of response cotyledon segments were surgically excised and inoculated into culture tubes containing MS[7] media Supplemented with 10 mg/l NAA and different concentrations of Endosulfan & Rogor (25, 50, 75,100, 150 and 200 ppm) and in combination of Endosulfan with Kitazin (10 +10, 20 + 20, 30 + 30, 50 + 50, 75 + 75 and 100 + 100 ppm) and Rogor + Kitazin (10 +10, 20 + 20, 30 + 30, 50 + 50, 75 + 75 and 100 + 100 ppm). After the addition of sucrose P^H of the media was adjusted to 5.6 – 5.8 with 0.1 N NaOH in HCl. The agar was added and heated gently with constant stirring till the added agar was dissolved and autoclaved for 15 min at 102 KPa. The cultures were incubated at 25 ± 2^0 C under fluoroscent white lights (1500 lux) maintained at 16:8 hr light and dark regime. Media with normal micronutrients and without the addition of pesticide was constituted as control. All the experiments were repeated thrice. The data was tabulated and analyzed statistically.

To ascertain the embryogenic nature of differentiating structures, cultured tissues were subjected to histological study. Callus bearing somatic embryos at different developmental stages was fixed in acetic acid: alcohol (1:3) then dehydrated in alcohol – Kylol series, embedded in paraffin wax, sectioned at 10 μ thickness and stained with heamatoxylin and basic fuschin.

RESULTS AND DISCUSSION

On the observations, placement of cotyledon on the media was also important for the somatic embryo response. It was noted that embryogenesis induction was more when abaxial surface of the cotyledon was placed touching the media compared to adaxial surface. The response of cotyledon was considered as the best explant for the induction of embryogenesis. MS media supplemented with NAA (10 mg/l) was suitable for the study of somatic embryogenesis. It was also significant the both induction and maturation of somatic embryos took place on the same media.

With the addition of Endosulfan the percentage of responding increased upto 100 ppm and then followed decrease in the response in higher concentrations of chemical. Control cultures shown 90% response and number of somatic embryos induced were 33.5. Somatic embryos frequency decreased in lower concentration of Endosulfan. The decreasing trend of induction was seen up to 75 ppm, however, there was a increase (24.6) noticed especially in 100 ppm and further decrease (18.4 & 14.5) observed in higher concentrations of 150 and 200 ppm respectively (TABLE 1). In Rogor added media both percentage of responding as well as induction of somatic embryos gradually decreased as their concentrations were increased. 200 ppm Rogor containing media showed lowest percentage of response (25.3). In both combined treated media of Endosulfan + Kitazin and Rogor + Kitazin, there was a decrease in both percentage of responding and number of somatic embryos as increasing their concentrations. Both combination treatments exhibited same response with regard to induction of somatic embryos.

Induction of somatic embryos by the incorporation of pesticides (Endosulfan, Rogor) to the media decreased as increasing concentrations both individually and in their combination with Kitazin. Exceptionally, in Endosulfan added media at 100 ppm equated the percentage of response with control cultures.

Induction of somatic embryos was much affected in combined treated (Endosulfan + Kitazin and Rogor + Kitazin) samples than their individual (Endosulfan, Rogor) treated cultures. It was important to note that, when compared to control cultures all the pesticides used individually and in combination showed less responding percentage and in an induction on somatic embryogenesis.

Torpedo and heart shaped somatic embryos were record frequently in control (Plate-I & Figure-a) and both globular and early torpedo shaped somatic embryos with hair on their surface were observed in (Rogor + Kitazin 20 + 20 ppm) treated samples (Plate-I & Figure-b). Mature and immature torpedo stage somatic embryos were observed in Rogor presence at lower concentration (25 ppm) (Plate-I & Figure-c). One of the somatic embryo with budding can be seen (arrow) in same concentration of Rogor. Cotyledonary stage somatic embryo was seen in Rogor at a concentration of 50 ppm (Plate-I & Figure-d).

Group of embryos at various developmental stages with long suspensor observed in proliferation media con-

FULL PAPER

TABLE 1 : Effect of Rogor and Endosulfan both individually and in their combination with Kitazin on the induction of somatic embryos

Concentration in ppm				No.of cultures maintained	Percentage of response	Average number of somatic embryos
Endosulfan	Rogor	Rogor + Kitazin	Endosulfan + Kitazin			
Control				30	90	33.5±0.1789
25				30	62.0	20.3 ±0.1642
50				30	70.4	22.5±0.1783
75				30	78.0	19.0±0.1152
100				30	90.0	24.6±0.1243
150				30	50.0	18.4±0.2146
200				30	25.0	14.5±0.1765
	25			30	80.0	36.5±0.1782
	50			30	65	24.4±0.1265
	75			30	64	21.5±0.1420
	100			30	42	17.4±0.2126
	150			30	38	16.0±0.1230
	200			30	25.3	14.6±0.1765
		10+10		30	58.4	18.6±0.2357
		20+20		30	52.0	17.2±0.2943
		30+30		30	42.2	16.0±0.1230
		50+50		30	35.0	12.5±0.1157
		75+75		30	28.3	12.0±0.1711
		100+100		30	16.0	10.0±0.1427
			10+10	30	50.0	14.5±0.1765
			20+20	30	42.2	10.0±0.1427
			30+30	30	30.0	8.3±0.1859
			50+50	30	250.	7.5±0.2582
			75+75	30	180.	7.1±0.1475
			100+100	30	15.0	5.0±0.2406

taining Endosulfan 25 ppm. Interestingly, various developmental stages of cluster of cotyledonary embryos at the end of 6th week was obtained from combined treatment of Endosulfan + Kitazin (20 + 20 ppm) added media (Plate-I & Figures e & f).

The individual treatment of pesticide Endosulfan 75 ppm remarkably induced series of embryos (5-6), which were confirmed their origion by histological study. All these series of embryos induced by Endosulfan showed synchronization with regard to maturity and germination. This future can be exploited for large scale production of somatic embryo. In some treated samples undescribed structures were also observed. Addition of pesticide to the media showed induction of only normal somatic embryos in all most all treated samples along with control, but there was no induction of abnormal somatic embryos were noted.

The matured somatic embryos developed from different samples were transferred to basal media MS + IBA (3 mg/l) and half MS media to view their regeneration capability. The individual pesticide treated somatic embryos only developed to plantlets and somatic embryos obtained from combined treated samples completely failed to regenerate. Basal and MS + IBA (3 mg/l) was suitable than half MS media, in which proper regeneration was not seen.

In the present study cotyledon explants gave best response. This is in conformity with other reports[8-11]. Francis Satyasealn and Jayachandran[12] also reported the importance of 2,4-D in combination with NAA in Somotic embryogenesis in *Solanum melongena*. The source of explants is important in determining regen-

PLATE – I

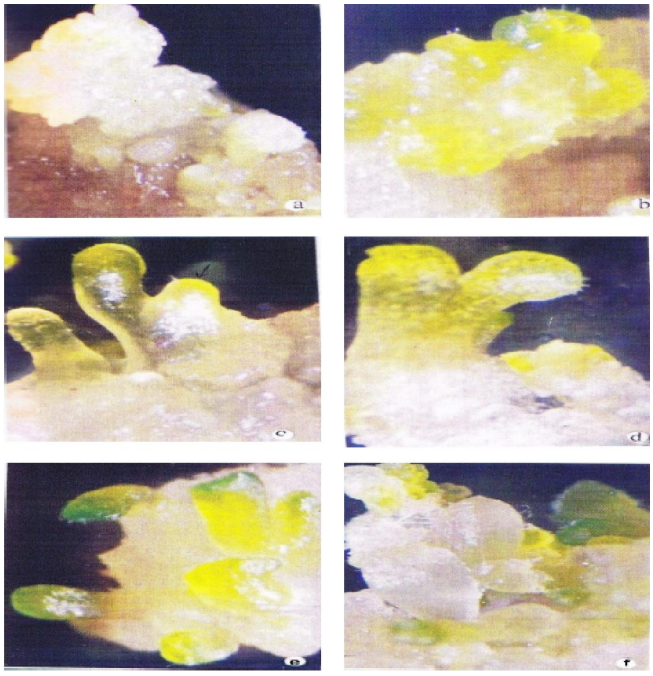

Plate - 1 : Somatic embryogenesis in S*olanum melongena* L.
Figure-a. Induction of proliferating embroygenic mass with globular embryos were observed in control samples (10mg/l NAA).
Figure-b. Globular and early torpedo embryos with hair on their surface in combined treated medium (Rogor+Kitazin-20+20ppm)
Figure-c. Mature and immature torpedo staged somatic embryos were observed in medium containing Rogor at 25 ppm. One of the somatic embryo with budding can be seen (arrow).
Figure-d. Cotyledonary stage somatic embryo was seen in media containing Rogor at 50 ppm.
Figures-e & f. Group of embryos at various developmental stages with long suspensor observed in proliferation media containing Endosulfan+Kitazin - 20+20ppm.

eration of plantlets via somatic embryogenesis has much potential for its use in plant regeneration[13-18]. Similar results were obtained in brinjal with heavy metals[19].

The pesticides Endosulfan & Rogor were absolutely necessary up to non toxic certain level and their high level presence leads to abnormal morphogenetic developments proved with the observations. Especially individual treatment of Endosulfan at suitable concentration (100 ppm) used in the present study to induce series of somatic embryos *in vitro* level.

ACKNOWLEDGEMENTS

The Authors are thankful to Head Department of Botany, Kakatiya University for providing necessary facilities to carryout the Research work.

ABBREVIATIONS

NAA – Naphthelene Acetic Acid, IBA – Indole Buteric Acid, MS – Murashige & Skoog's.

REFERENCES

[1] F.T.Davies, S.A.Duray; J.Env.Hort., **1**, 181 **(1992)**.
[2] K.M.Azam, S.A.Razvi, Z.Ali, A.A.Al-Raeesi; Indian J.Plant Prot., **25**, 36 **(1997)**.
[3] S.Bhagat, B.K.De; Enviorn.& Ecol., **19**, 853 **(2001)**.
[4] B.Jerlin; J.Ecotoxical Eniviron.Monito., **11**, 209 **(2001)**.
[5] S.Gleddie, W.Keller, G.Setterfiekd; can J.Bot., **61**, 656 **(1983)**.
[6] H.Mastsuoka, K.Hinata; J.Exp.Bot., **30**, 363 **(1979)**.
[7] T.Murashige, F.Skoog; Physiol.Plant., **15**, 473 **(1962)**.
[8] M.Carola Fiore, Trabace, Francesco Sunseri; Plant Cell Rep., **16**, 295 **(1997)**.
[9] K.Srinivasu, S.K.Malik, P.Anand Kumar, R.P.Sharma; Plant Cell Rep., **17**, 294 **(1998)**.
[10] L.George, S.Eapen; Plant Cell Report. **13**, 417 **(1994)**.
[11] G.Jenings, R.Bronner, G.Hahne; Plant Cell Rep., **15**, 200 **(1995)**.
[12] M.Francis Satyasealan, F.Jayachandran; Department of Botany, Kerala, India., **2**, 58 **(2000)**.
[13] P.V.Ammirato; Plant tissue and cell culture, Liss, New York., 57, **(1987)**.
[14] S.P.Sagare, K.Suhasini, K.V.Krishna Murthy; Plant Cell Rep., **12**, 652 **(1993)**.
[15] A.Dave, A.Batra; plant tissue and cell culture., **3**, 51 **(1994)**.
[16] A.S.Rani, G.M.Reddy; Plant tissue culture and molecular biology, Hyderabad India., 29, **(1997)**.
[17] P.Venkatachalam, P.B.Kavikishore, N.Jayabalan; Curr.Sci., **77**, 271 **(1997)**.
[18] Ashok Chaudhury, O.U.Rongda; Plant cell tissue Org.Cult., **60**, 113 **(2000)**.
[19] P.Neelima, K.Jaganmohan Reddy, C.H.Chandra Shekar; L.Plant Cell Biotechnology and Molcular Biology. **5(3&4)**, 129 **(2004)**.